Genetic evaluation of the Flathead Chub (*Platygobio gracilis*)

Technical Report
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Introduction

The Flathead Chub, *Platygobio gracilis* is composed of two subspecies *Platygobio g. gracilis*, and *Platygobio g. gulonella*. The nominal subspecies is thought to be a large river form while *P. g. gulonella* is more characteristic of creeks and small rivers (Olund and Cross, 1961). The two subspecies are distinguished from one another on the basis of overall body shape, head profile, orbit size, degree to which the fins are falcate, lateral line scales, pectoral ray counts, and post-weberian vertebrae (Olund and Cross, 1961). The nominal subspecies is distributed in the Mackenzie and Saskatchewan basins, and the mainstem of the Missouri and Mississippi rivers. *Platygobio gracilis gulonella* is confined to the Pecos, upper Rio Grande, Arkansas, North Platte and some tributaries to the upper Missouri rivers (Olund and Cross, 1961). A modified version of the distribution map from Olund and Cross (1961) is shown in Fig. 1. As can be seen in this map, many populations are labeled as “intergrades” between the two subspecies. This is based on a perceived intermediacy of diagnostic characters in these populations. For the sake of consistency with respect to existing taxonomy (Page and Burr, 1991), we have maintained the designation of two subspecies, and “intergrade populations” throughout the remainder of this study.

While historically this species was among the most abundant minnows in the turbid waters of the Missouri River (Bailey and Allum, 1962), recent surveys of flathead chubs have shown a dramatic decline in their abundance (Pflieger, 1997). Currently, this species is listed as of "particular concern" by both regions 3 and 6 of the U.S. Fish and Wildlife Service (FWS). The 1997 Flathead Chub Status Assessment of the FWS indicated that genetic analysis of the two nominal subspecies was an essential piece of missing data that

would help clarify the status of this species so that well informed decisions pertaining to its management and potential listing could be made. To date however no such study has been completed, and information regarding genetic variation within flathead chubs is lacking. The following report details the results of a genetic analysis of 20 individuals of flathead chub from throughout their range based on a dataset composed of 950 base pairs of aligned sequence data from the mitochondrial cytochrome *b* gene.

Methods

Cytochrome *b* sequences (cyt *b*) were generated for 20 individuals of *Platygobio gracilis* representing eight populations from throughout their known range (Fig. 1). This included nine individuals of *P. gracilis gracilis*, two individuals of *P. gracilis gulonella*, and nine individuals from the hypothesized zone of intergradation. A complete listing of all sample localities is provided in the Material Examined section at the end of the report. Additionally, one individual of *Hybopsis amblops* and one individual of *H. winchelli* were sequenced for use as functional ingroup taxa. This enabled a limited test of *Platygobio* monophyly. Sequence data is available from RMW upon request by email to wood2@slu.edu. DNA was extracted from muscle tissue using the Puregene DNA Isolation Kit (Gentra Systems, Inc.). The cyt *b* gene was amplified with primers located in flanking $tRNA^{GLU}$ and $tRNA^{THR}$ genes (Schmidt and Gold, 1993). PCR was run for 23 cycles (50 µl total volume), with primers in equal concentration to produce a double stranded product. Denaturation, annealing, and extension temperatures and times were: 94 C, 1 min; 48 C, 1 min; and 72 C, 2 min, respectively. All PCR reactions contained 2.5 mM concentrations of $MgCl_2$, other conditions were as recommended by the manufacturer. The

double-stranded PCR product was purified in a Millipore Ultra Free Spin Filter, following manufacturer's instructions, and then resuspended in 20 µl of ddH_2O. Three µl of cleaned double stranded product was used in subsequent sequencing reactions.

The cyt *b* gene (950 bp) was sequenced in two segments using dye--terminated cycle sequencing chemistry, sequences being read on a Beckman CEQ 2000 sequencer. Two primers were used for sequencing reactions: the flanking primer in the tRNA--Glu region, used in PCR above (Schmidt and Gold, 1993), and an internal primer designed in the Wood lab for sequencing minnows LH2 (5' TGR GGH CAR ATR TCV TTY TG 3,' where y is c or t; r is a or g; v is a, c, or g; and h is a, t, or c; 20mer; position 403--422). Sequencing conditions included a dwell time of 1 min at 96 C and denaturation, annealing and extension temperatures and times of 96 C, 10 sec; 50 C, 4 sec; 60 C, 4 min, respectively. The cyt *b* gene sequences were aligned to previously generated sequences from two individuals of the genus *Notropis* using ClustaIX (vers. 1.64b, Thompson et al., 1997). Genetic distances (p-distance) between individuals and populations were calculated using PAUP* (vers. 4.Ob2a, D.L. Swofford, PAUP: phylogenetic analysis using parsimony, Smithsonian Institution, Washington, DC, 1993, unpubl.). Average sequence divergence within and between populations was calculated in a Microsoft Excel spreadsheet.

Results

The 20 individuals of *Platygobio gracilis* exhibited average within population sequence divergence ranging from a low of 0.3% between individuals from the Loup River (PG07-09) to a high of 2.2% between individuals sampled from the Iowa tributaries to the Missouri River (Samples GC01, MC01, and WC01). Average between population

sequence divergence ranged from a low of 0.4% between the Niobrara and Loup river samples and Loup and Cheyenne river samples, to a high of 5.9% between the Canadian River sample and the sample from the Rio Grande. Pairwise distances shown as: absolute numbers of nucleotide substitutions between taxa are shown in Table 1; percent sequence divergence between taxa in Table 2; and, average within sample and between sample divergence in Table 3.

Phylogenetic analysis of the 950 aligned bases for the 20 individuals (plus four outgroup taxa) sequenced in this study resulted in 18 equally most parsimonious trees at 595 steps (C.I. = 0.848, RC = 0.645). A strict consensus of these 18 trees is shown in Figure 2. A phylogram (tree where branch lengths are proportional to the number of nucleotide substitutions) for one of these trees is shown in Figure 3. As can be seen there is very little divergence among taxa and most individuals fail to group by either geographic location or existing subspecific taxonomy.

Discussion

It is interesting that the highest level of sequence divergence observed between any samples in this study was between two individuals of the same subspecies, *P. gracilis gulonella* from the Rio Grande and Canadian rivers. These samples differ by nearly 6% sequence divergence. This is a rather pronounced level of genetic variation between two populations of the same subspecies given the conservative nature of the cyt *b* marker. There are a number of cases in fishes where well accepted **species** do not exhibit this much variation at the cyt *b* locus. A few examples I have worked on include *Notropis blennius* and *N. potteri* which are only separated by 3.9% divergence (Raley and Wood, in press),

and at least 3 pairs of darter taxa including *Etheostoma rubrum* and *E. moorei* and *E. tippecanoe* and *E. denoncourti* that differ by less than 5% sequence divergence (Kinziger et al., 2001; Wood, unpubl. Data).

Samples of *P. g. gracilis* (labeled populations A-E, and H in Table 3) exhibited only moderate variation when considered collectively. The observed sequence divergence ranged from a low of 0.4% divergence between the Niobrara and Loup river samples, and Loup and Cheyenne river samples to a high of 2.5% divergence between the Peace River sample (Alberta, Canada) and the sample from the Iowa tributaries to the Missouri River. Upon closer inspection however, a slightly more interesting pattern emerges. The Peace River sample (Mackenzie River System) differs from all populations in the Missouri River System by sequence divergences ranging from a low of 0.8% divergence to a high of 2.5% divergence. While this is not as pronounced as the variation observed between the Rio Grande sample and the Canadian River sample, it is by no means trivial. The populations in the Mackenzie and Saskatchewan basins (represented in this study only by individuals from the Peace River) are likely recent dispersers into these systems following the retreat of glaciers at the close of the Pleistocene. The close phylogenetic affinity of the Peace River and Missouri River samples (in fact, the Peace River samples are not supported as monophyletic with respect to populations from the Missouri) and the observed level of sequence divergence seems consistent with this interpretation.

The final piece of data that seems notable is the moderate level of divergence observed between the tributaries to the Missouri River in Iowa and all other Missouri River tributary populations. These populations differed by between 1.8 and 2.1% sequence divergence. This coupled with the observation that this is the only tributary system east of

the Missouri River sampled (within the Missouri River System, flathead chubs are rare in eastern tributaries) indicates that these populations merit further study and should be treated cautiously until more is known about their affinity with respect to remaining flathead chub populations.

Summary and Recommendations

In light of these data it seems appropriate to treat the populations from the Rio Grande and Canadian river systems as separate management units. This is supported by the rather pronounced level of genetic variability (5.9% sequence divergence) observed between samples from these localities. Second, although the population from the Peace River (Mackenzie River System) fails to form a monophyletic group in parsimony analysis of the data, the genetic distances separating this population from Missouri River populations of flathead chub warrants its treatment as an additional management unit. Third, given the moderate level of sequence divergence observed between the Iowa tributary populations and all other populations from the Missouri River system, we tentatively recommend that these be considered a third management unit and recommend that these populations are in need of additional study. Finally, with respect to remaining populations from the Missouri River system, although they exhibit a rather low level of genetic differentiation at the cyt *b* locus, the rather pronounced morphological differences between the two subspecies within this system summarized by Olund and Cross (1961), suggests that a very cautious approach should be used if any future management activities are to include either captive propagation or transplantation of any fishes from these populations.

Material Examined

Platygobio gracilis gracilis. **Peace River Drainage**. Sample PG 01-03. Smoky River at ALB Hwy 34, 9.5 mi W of Debolt, Alberta, Canada. UAIC 11218.02. June 15, 1995. **Missouri River Drainage**. Sample PG 04-06, Niobrara River at NB Hwy 12 (W side), 1,3 mi WSW of Niobrara, Knox Co., Nebraska. UAIC 11168.06. July 9, 1995. Sample GC 01. Graybill Creek at Cold Spring State Park, Pottawattamie Co., Iowa. July 19, 2000. Sample MC 01. Mosquito Creek at Cold Springs State Park, Montgomery Co., Iowa. August 10, 2000. Sample WC 01. Walnut Creek at Cold Springs State Park, Montgomery Co., Iowa. August 1, 2000. **Hypothesized Intergrades**. **Missouri River Drainage**. Sample PG 07-09. Loup River (Tributary to Platte) at US Hwy 81/30 on S side of Columbus, at Pawnee Park, Platte Co., Nebraska. UAIC 11220. June 9,1995. Sample PG10-12. Cheyenne River at SD Hwy 144, 3 mi ESE of Creston, Pennington Co., South Dakota. UAIC 11238. June 11, 1995. Sample PG13-15. Little White River at US Hwy 83, 2.2 mi N of White River, Mellette Co., South Dakota. UAIC 11169.03. June 11, 1995.

Platygobio gracilis gulonella. **Mississippi River Drainage**. Sample PG 16. Canadian River at US Hwy 54 in Logan, Quay Co., New Mexico. UAIC 12303.01. May 30, 1994. **Rio Grande Drainage**. Sample PG 17. Rio Grande River at NM Hwy 44, N. of Bernalillo. UAIC 12304.01. June 1, 1994.

Table 1. Absolute number of pairwise nucleotide differences among taxa.

		1	2	3	4	5	6	7	8	9	10	11	12
1	AMBLOPS01	-											
2	HWCR01	72	-										
3	pg01	139	143	-									
4	pg02	141	145	3	-								
5	Pg03	151	156	14	17	-							
6	pg04	139	141	3	6	17	-						
7	pg05	138	142	3	6	17	2	-					
8	pg06	145	146	6	9	20	7	7	-				
9	pg07	139	143	2	5	16	3	3	8	-			
10	pg08	139	141	2	5	16	1	1	6	2	-		
11	pg09	138	144	1	4	15	4	4	7	3	3	-	
12	pg10	141	143	4	7	18	7	7	10	6	6	5	-
13	pg11	140	142	1	4	15	2	2	5	3	1	2	5
14	pg12	140	141	3	6	17	4	4	5	5	3	4	7
15	pg13	132	126	8	10	22	8	8	12	9	7	9	12
16	pg14	140	144	4	7	18	7	7	10	6	6	5	8
17	pg15	139	143	0	3	14	3	3	6	2	2	1	4
18	pg16	171	173	41	43	55	42	42	47	41	41	42	45
19	pg17	146	148	22	25	34	23	25	26	24	24	23	25
20	GC01LA	102	108	8	10	22	10	10	14	8	9	8	11
21	MC01LA	102	108	16	18	30	18	18	19	16	17	16	19
22	WC01LA	129	123	14	16	28	14	14	18	14	13	15	18

Absolute number of pairwise distances (continued)

		13	14	15	16	17	18	19	20	21	22
13	pg11	-									
14	pg12	2	-								
15	pg13	7	9	-							
16	pg14	5	7	11	-						
17	pg15	1	3	8	4	-					
18	pg16	42	44	10	45	41	-				
19	pg17	23	23	21	26	22	56	-			
20	GC01LA	9	11	14	7	8	8	19	-		
21	MC01LA	17	19	18	15	16	16	25	13	-	
22	WC01LA	13	15	15	17	14	16	26	14	17	-

Table 2. Percent sequence divergence (uncorrected) among taxa.

		1	2	3	4	5	6
1	AMBLOPS01	-					
2	HWCR01	0.07587	-				
3	pg01	0.14647	0.15068	-			
4	pg02	0.14858	0.15279	0.00316	-		
5	Pg03	0.15963	0.16492	0.01479	0.01796	-	
6	pg04	0.14647	0.14858	0.00316	0.00632	0.01796	-
7	pg05	0.14542	0.14963	0.00316	0.00632	0.01796	0.00211
8	pg06	0.15279	0.15385	0.00632	0.00948	0.02113	0.00738
9	pg07	0.14647	0.15068	0.00211	0.00527	0.01690	0.00316
10	pg08	0.14647	0.14858	0.00211	0.00527	0.01690	0.00105
11	pg09	0.14542	0.15174	0.00105	0.00421	0.01584	0.00421
12	pg10	0.14858	0.15068	0.00421	0.00738	0.01901	0.00738
13	pg11	0.14752	0.14963	0.00105	0.00421	0.01585	0.00211
14	pg12	0.14752	0.14858	0.00316	0.00632	0.01796	0.00421
15	pg13	0.16236	0.15473	0.00985	0.01232	0.02715	0.00987
16	pg14	0.14752	0.15174	0.00421	0.00738	0.01901	0.00738
17	pg15	0.14647	0.15068	0.00000	0.00316	0.01479	0.00316
18	pg16	0.18019	0.18230	0.04320	0.04531	0.05813	0.04426
19	pg17	0.15419	0.15624	0.02323	0.02640	0.03601	0.02428
20	GC01LA	0.14659	0.15540	0.01147	0.01433	0.03163	0.01433
21	MC01LA	0.15730	0.16676	0.02432	0.02738	0.04591	0.02735
22	WC01LA	0.16492	0.15714	0.01792	0.02045	0.03594	0.01794

		7	8	9	10	11	12	13	14
7	pg05	-							
8	pg06	0.00738	-						
9	pg07	0.00316	0.00843	-					
10	pg08	0.00105	0.00632	0.00211	-				
11	pg09	0.00421	0.00738	0.00316	0.00316	-			
12	pg10	0.00738	0.01054	0.00632	0.00632	0.00527	-		
13	pg11	0.00211	0.00527	0.00316	0.00105	0.00211	0.00527	-	
14	pg12	0.00421	0.00527	0.00527	0.00316	0.00421	0.00738	0.00211	-
15	pg13	0.00987	0.01476	0.01108	0.00863	0.01109	0.01478	0.00862	0.01109
16	pg14	0.00738	0.01054	0.00632	0.00632	0.00527	0.00843	0.00527	0.00738
17	pg15	0.00316	0.00632	0.00211	0.00211	0.00105	0.00421	0.00105	0.00316
18	pg16	0.04426	0.04953	0.04320	0.04320	0.04426	0.04742	0.04426	0.04636
19	pg17	0.02640	0.02745	0.02534	0.02534	0.02428	0.02641	0.02428	0.02428
20	GC01LA	0.01433	0.02012	0.01148	0.01289	0.01147	0.01570	0.01289	0.01576
21	MC01LA	0.02735	0.02883	0.02430	0.02583	0.02432	0.02878	0.02583	0.02885
22	WC01LA	0.01794	0.02304	0.01791	0.01665	0.01919	0.02303	0.01664	0.01921

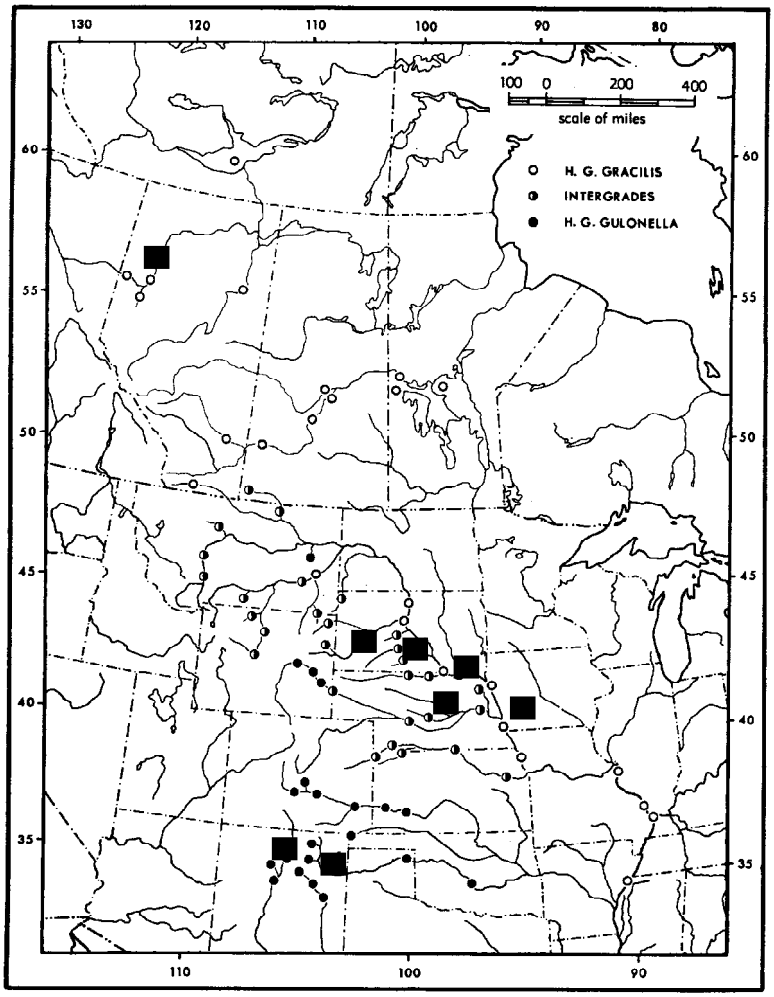
		15	16	17	18	19	20	21	22
15	pg13	-							
16	pg14	0.01358	-						
17	pg15	0.00985	0.00421	-					
18	pg16	0.01236	0.04742	0.04320	-				
19	pg17	0.02590	0.02745	0.02323	0.05915	-			
20	GC01LA	0.01999	0.01004	0.01147	0.01158	0.02724	-		
21	MC01LA	0.02709	0.02281	0.02432	0.02439	0.03814	0.01998	-	
22	WC01LA	0.01916	0.02176	0.01792	0.02044	0.03331	0.02018	0.02603	-

Table 3. Average within population sequence divergence (along the diagonal) and between population sequence divergence (lower half of matrix) separating populations. Population Codes: **A**. **S**ample PG 01-03, Peace River. **B.** Sample PG 04-06, Niobrara River. **C**. Sample PG 07-09, Loup River. **D**. Sample PG10-12, Cheyenne River. **E**. Sample PG13-15, Little White River. **F**. Sample PG 16, Canadian River. **G**. Sample PG 17, Rio Grande River. **H**. Sample GC 01, Graybill Creek; MC 01, Mosquito Creek; WC 01, Walnut Creek.

	A	B	C	D	E	F	G	H
A	0.012							
B	0.010	0.006						
C	0.008	0.004	0.003					
D	0.009	0.005	0.004	0.005				
E	0.011	0.008	0.006	0.007	0.009			
F	0.049	0.046	0.044	0.046	0.034	0.000		
G	0.029	0.026	0.025	0.025	0.026	0.059	0.000	
H	0.025	0.021	0.018	0.021	0.019	0.019	0.033	0.022

Figure 1. Distribution map of *Platygobio gracilis* (modified from Plate 21 of Olund and Cross, 1961). Solid black squares indicate sampling localities for specimens included in the genetic analysis. See Material Examined.

PLATE 21



Distribution of collections examined.

Figure 2. Strict consensus tree of 18 most parsimonious resolutions of the data. Tree length = 595 steps (C.I. = 0.848, RC = 0.645).

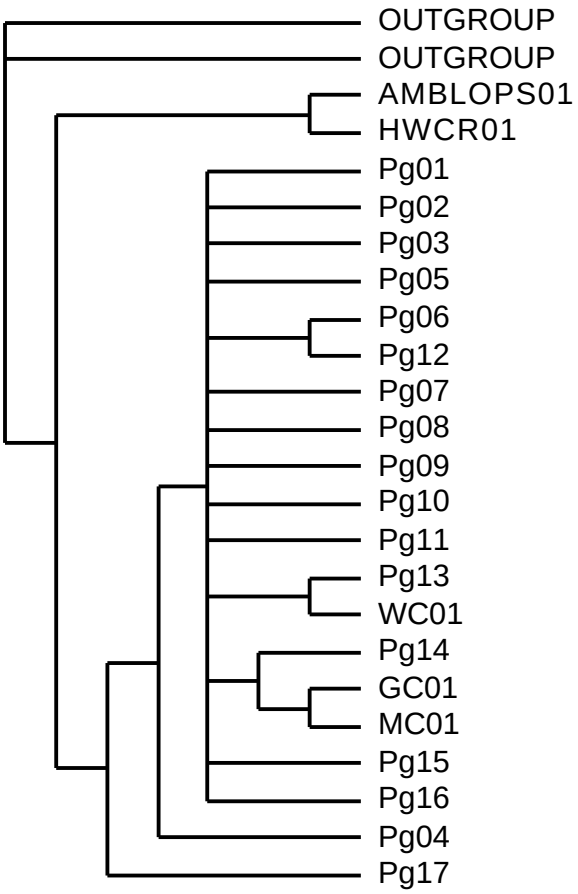
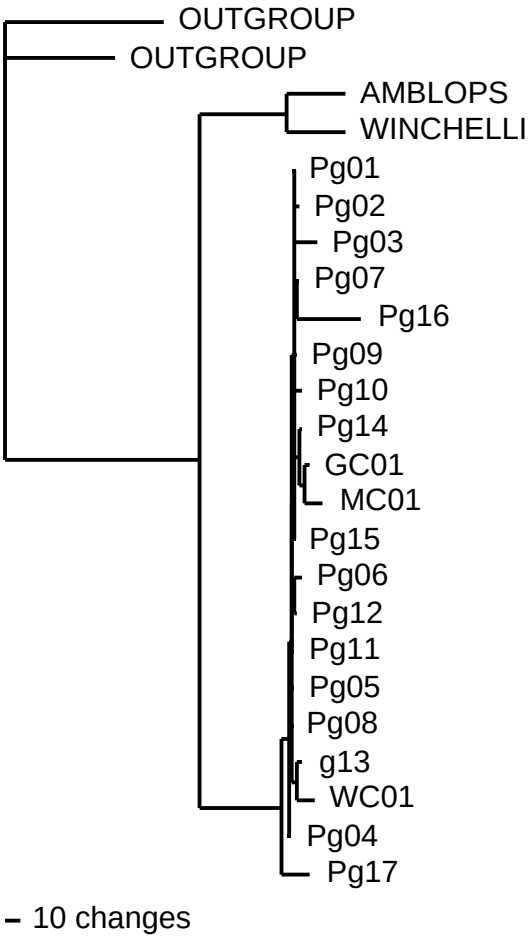


Figure 3. Phylogram of one of 18 most parsimonious resolutions of the data. This is provided to illustrate the overall lack of divergence among flathead chubs. Notable exceptions include the sample from the Rio Grande Drainage and the sample from the Mississippi River Drainage (see discussion).



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